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Physicochemical Properties of Spicy Lactic Fermented Tigernut-milk Drink Monitored during Ambient and Refrigeration Temperature Storage

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

The development of non-dairy fermented products is fast gaining acceptability due to its unique benefits. Several factors such as storage conditions, processing methods and constituent of such products usually affect its physicochemical properties. Tigernut-milk drink fermented by mixed culture of lactic acid bacteria (LAB) isolated from 'ogi' identified as *Lactobacillus plantarum, L. acidophilus, L. brevis* and *Streptococcus thermophilus* using API 50 CHL test kit was separately spiced with 3%, 5% ginger; 3%, 5% garlic. Each tigernut-milk drink was stored at ambient (28±2°C) and refrigeration (4±2°C) temperature for 12 weeks. Non-spiced lactic fermented tigernut-milk drink was the control. At 4 weeks interval, ethanol content, calorific value and total soluble solids (TSS) in the drink were monitored. During ambient and refrigeration temperature storage, there was increase in ethanol content of the spiced and non-spiced lactic fermented tigernut-milk drinks. Maximum ethanol content of spicy lactic fermented tigernut-milk drink stored at 4±2°C and 28±2°C was 0.73 g/L and 0.96 g/L, respectively. At both storage conditions, non-spiced lactic fermented tigernut-milk drink had the highest ethanol content 1.76 g/L. There was reduction in calorific value with few exceptions and continuous reduction in TSS which ranged between (245.08-134.41 Kcal) and (14.31-5.29 °B) in the spicy lactic fermented tigernut-milk drinks during storage at 28±2°C and

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4±2°C, respectively. This study revealed that calorie content and total soluble solids in ginger and garlic spiced lactic fermented tigernut-milk drinks considerably reduced during the period of storage at ambient and refrigeration temperature with few exceptions while ethanol content of the drinks consistently increased.

Keywords: Lactic acid bacteria; tigernut-milk drink; fermentation; flavouring agents; storage temperature.

1. INTRODUCTION

Over the years, researchers have made tremendous improvement in the quality of dairy products in terms of nutritional content, safety, sensory characteristics and shelf life of the products to meet the requirements of millions of people in different age brackets, cultural background and religious beliefs. However, certain individuals prefer non-dairy products to popular dairy products because of allerginicity, lactose intolerance, cholesterol and saturated fat content. In recent times, efforts through research geared towards improving the quality of non-dairy products had recorded successes but more studies are still required in order to match the level of improvement recorded in dairy products [1,2,3,4].

Tigernut milk could be regarded as imitation milk prepared from tigernut tubers. It is the most popular product derived from tigernut. In Spain, Ghana and Northern Nigeria, tigernut-milk is known as “chufa de horchata’, ‘atadwe’ and ‘kunu-aya’, respectively. Tigernut-milk can be consumed wholly or combined with other liquid beverages. Generally, tigernut-milk drink is regarded as a non-alcoholic drink rich in nutrients [5]. When tigernut-milk drink is subjected to fermentation, some quantity of ethanol is produced as a result of microbial activities involved in the fermentation process. Tigernut-soymilk extract (TSME) is a source of high quality energy, protein, minerals and vitamins suitable for children, grown-ups and sports men [6,7]. Tigernut milk is an energetic drink because of its calorie content [8,9]. According to [10], total energy value of tigernut-milk drink is from its fat content.

Tigernut-milk drink can be subjected to fermentation using yoghurt starter and probiotics to produce tigernut-milk yoghurt and probiotic product, respectively. Wakil [10] identified some lactic and non-lactic bacteria in tigernut-milk drink undergoing fermentation. ‘Ogi’ is an acid fermented gruel indigenous to Nigeria. It is also consumed in few other African countries. Ogi is a fermented gruel prepared using maize, millet or sorghum. Lactic acid bacteria dominate microorganisms involved in the fermentation process. The fermentation of tigernut-milk drink is responsible for the release of alcohol in the form of ethanol which gives the product its characteristic flavour and also functions as a preservative to the fermented product [11, 12,13].

There is dearth of information about the quantity of ethanol in lactic fermented tigernut-milk drink. Ethanol is known as grain alcohol or drinking alcohol [14]. Globally, alcoholic drink is intentionally consumed by millions of people. Alcohol is moderately consumed by taking part in Italian diets. Despite numerous disadvantages of excessive intake of alcohol to the human body, there are few health benefits derived by the body when products that contain alcohol are moderately consumed. Recent studies revealed that moderate alcohol consumption gives the human heart some level of protection against some diseases. Cough syrups and some medications are produced using alcohol. Ethanol is a volatile compound. It is a very effective olfactory and trigeminal stimulus used for the production of dairy products such as eggnog and rum-raisin ice cream. The purpose of adding ethanol to some products is to enhance flavour and organoleptic attributes of such products as well as function as a preservative [15].

A study to determine the effect of ethanol on yoghurt culture- *Lactobacillus delbrueckiis sp. bulgaricus* (LB-12) and *Streptococcus thermophilus* (ST-MS) was carried out by [15]. In a related study, [16] monitored ethanol content of tigernut wine at different fermentation periods. Incorporating ginger and garlic into most products is for the purpose of adding flavour as well as extend the shelf life of such products. To this end, this study is aimed at determining the effect of storage temperature and flavouring agents (ginger, garlic) on some physicochemical properties (total soluble solids, ethanol and

calorie content) of lactic fermented tigernut-milk drink.

2. MATERIALS AND METHODS

2.1 Fermentation of Tigernut-milk Drink Using Lactic Starter Culture

Tigernut-milk drink was prepared using a similar method described by [17]. The product was allowed to ferment for 72 h at 45°C after 2% (w/v) mixed culture of *Streptococcus thermophilus, Lactobacillus plantarum, L. acidophilus* and *L. brevis* isolated from 'ogi' was inoculated into the drink using the method described by [18,19].

2.2 Preparation of Lactic Fermented Tigernut-milk Drink Spiced with Ginger and Garlic

Ginger and garlic were purchased from Oshodi market, Lagos state. The bark of ginger and garlic were removed before it was crushed into a powder. Three gram and five gram of the crushed powder were separately mixed with 100 ml distilled water. The two concentrations, 3% (w/v) and 5% (w/v) ginger and garlic were pasteurized at 72°C for 15 min. The freshly prepared lactic fermented tigernut-milk drink was separately spiced with 3% (w/v) ginger, 5% (w/v) ginger, 3% (w/v) garlic and 5% (w/v) garlic.

2.3 Physicochemical Properties of Spicy Lactic Fermented Tigernut-milk Drink during Storage

The freshly prepared ginger and garlic spiced lactic fermented tigernut-milk drinks were stored at ambient (28±2°C) and refrigeration (4±2°C) temperature for 12 weeks. At 4 weeks interval, total soluble solids, calorific value and ethanol content of the spicy lactic fermented tigernut-milk drinks was determined.

2.3.1 Determination of total soluble solids in spicy lactic fermented tigernut-milk drink

The total soluble solids in ginger and garlic spiced lactic fermented tigernut-milk drink stored at 28±2°C and 4±2°C were determined in duplicate using Abbe 60 Refractometer and the results expressed as degree of brix (°Brix) using the procedure described by [20].

2.3.2 Determination of calorific value of spicy lactic fermented tigernut-milk drink

The calorific value per 100 ml lactic fermented tigernut-milk drink spiced with ginger and garlic was calculated using the system of Atwater (in Kcal) equation: Energy (kcal) = (3.36 x % protein) + (3.60 x % carbohydrate) + (8.37 x % fat) reported by [21].

2.3.3 Determination of ethanol content of spicy and non-spicy lactic fermented tigernut-milk drink

Roche-Biopharm method for determination of ethanol content of alcoholic beverages was used to determine the ethanol content in spiced and non-spiced lactic fermented tigernut-milk drink.

3. RESULTS AND DISCUSSION

Physicochemical changes as a result of microbial activities and chemical reactions usually occur during storage of fermented liquid beverages. These changes directly or indirectly affect the overall quality of the product. Fig. 1 shows the ethanol content of spicy and non-spicy lactic fermented tigernut-milk drinks stored at ambient (28±2°C) and refrigeration (4±2°C) temperature for 12 weeks. During refrigeration temperature storage, this study revealed that ethanol content of lactic fermented tigernut-milk drink spiced with 3%, 5% ginger; 3%, 5% garlic continuously increased from 0.0 g/L (at Week 0) to 0.46 g/L, 0.49 g/L, 0.73 g/L and 0.51 g/L, respectively at Week 12. A similar result trend was also observed during storage of 3%, 5% ginger; 3%, 5% garlic spiced lactic fermented tigernut-milk drink at ambient temperature which witnessed increase in ethanol content from 0.0 g/L (at Week 0) to 0.96 g/L, 0.58 g/L, 0.84 g/L and 0.81 g/L, respectively at Week 12. Increase in ethanol content of lactic fermented tigernut-milk drinks spiced with ginger and garlic during ambient and refrigeration temperature storage could be attributed to increase in viable count of mainly acid tolerant bacteria which is capable of releasing CO_2, ethanol, lactic acid and acetic acid into the spiced and non-spiced lactic fermented tigernut-milk drinks. According to [22], some *Lactobacilli* are heterofermenters. These heterofermenters produce equal molar amount of lactate, CO_2 and ethanol as fermentation end products.

The result in Fig. 1 revealed that 1.76 g/L of ethanol in non-spiced lactic fermented tigernut-milk drink stored at refrigeration (4±2°C) temperature at Week 12 was higher than ethanol content 0.46 g/L, 0.49 g/L, 0.73 g/L and 0.51 g/L in 3%, 5% ginger; 3%, 5% garlic spiced lactic fermented tigernut-milk drinks subjected to same storage condition, respectively. Similarly, ethanol content 1.76 g/L of non-spiced lactic fermented tigernut-milk drink stored at ambient temperature (28±2°C) at Week 12 was also higher than ethanol content 0.96 g/L, 0.58 g/L, 0.84 g/L and 0.81 g/L in 3%, 5% ginger; 3%, 5% garlic spiced lactic fermented tigernut-milk drinks subjected to same storage condition, respectively. In fact, the ethanol content of spiced lactic fermented tigernut-milk drinks monitored at 4 weeks interval for 12 weeks was consistently lower than ethanol content of non-spiced lactic fermented tigernut-milk drink. Lower ethanol content of lactic fermented tigernut-milk drinks spiced with ginger and garlic when compared with the non-spiced lactic fermented tigernut-milk drink could be as a result of ginger and garlic was added to the drink before storage. It could also be that population of lactic culture in tigernut-milk drinks spiced with ginger and garlic was lower than non-spiced lactic fermented tigernut-milk drink because of antimicrobial substances present in ginger and garlic which probably reduced rate of lactic fermentation to some extent that resulted in lower ethanol production. In a related study, [23] reported that lactic acid bacterial count in non-spiced lactic fermented tigernut-milk drink stored at ambient (28±2°C) and refrigeration temperature (4±2°C) for 12 weeks was higher than lactic fermented tigernut-milk drinks spiced with ginger and garlic with few exceptions at week 0.

According to [24], lactic acid bacteria can tolerate up to 20% ethanol content in beverages. In fruit mashes, some strains of *Lactobacillus plantarum* and *Lactobacillus suebicus* can survive in fermented beverages that contain approximately 12% and 14% ethanol, respectively. Other *lactobacillus* sp. that can tolerate the presence of ethanol in fermented beverages includes *Lactobacillus brevis*, *Lactobacillus fructivorans* and *Lactobacillus hilgardii*. In a related study, [14] reported that *Streptococcus thermophilus* has the ability to grow in fermented beverages that contain little quantity of ethanol. This result is in agreement with a similar study carried out by [14] in a study that involved incorporating ethanol into yoghurt. The authors reported that ethanol added to the product did not adversely affect the viability of *Lactobacillus bulgaricus* or *Streptococccus thermophilus.*

Taking into consideration the significant differences in ethanol content of each lactic fermented tigernut-milk drink spiced with a particular concentration of ginger or garlic monitored at 4 weeks interval during storage at ambient (28±2°C) and refrigeration temperature (4±2°C), it can be inferred that both storage conditions influenced ethanol content of the drinks. However, both storage conditions did not significantly affect the ethanol content of non-spiced lactic fermented tigernut-milk drink. Fig. 1 revealed that the quantity of ethanol in spiced and non-spiced lactic fermented tigernut-milk drinks stored at ambient temperature was higher than ethanol content of a similar product stored at refrigeration temperature. It could be that ambient temperature storage (28±2°C) was more conducive than refrigeration temperature storage (4±2°C) for lactic culture in spicy lactic fermented tigernut-milk drink to increase her population which translated into increased fermentation process and yielded more quantity of ethanol as one of its end product. According to [25], viability of probiotic bacteria is significantly affected by fermentation temperature. Optimum temperature for most probiotic bacteria is 37°C.

Table 1 depicts the calorific value of ginger and garlic spiced lactic fermented tigernut-milk drinks stored at ambient (28±2°C) and refrigeration (4±2°C) temperature for 12 weeks. This study revealed that there was continuous reduction in calorific value per 100 ml lactic fermented tigernut-milk drinks spiced with ginger and garlic during ambient and refrigeration temperature storage with few exceptions. Continuous reduction in calorific value of ginger and garlic spiced lactic fermented tigernut-milk drinks could be as a result of breakdown of carbohydrate, sugar and lipid in the tigernut-milk drinks by lactic culture in the drinks [26,27,28]. In a related study, [29] reported that there was reduction in protein content (7.4-6.8%), fat content (6.1-5.6%) and sugar content (11.0-2.0%) of fermented tigernut milk drink stored at 4°C for 14 days. During storage of a similar drink at 32°C for 14 days, [29] reported there was also reduction in protein content (7.6-6.6%), fat content (6.1-5.4%) and sugar content (11.0-1.0%). During the period of storage, this study revealed that there were significant differences in calorie content per

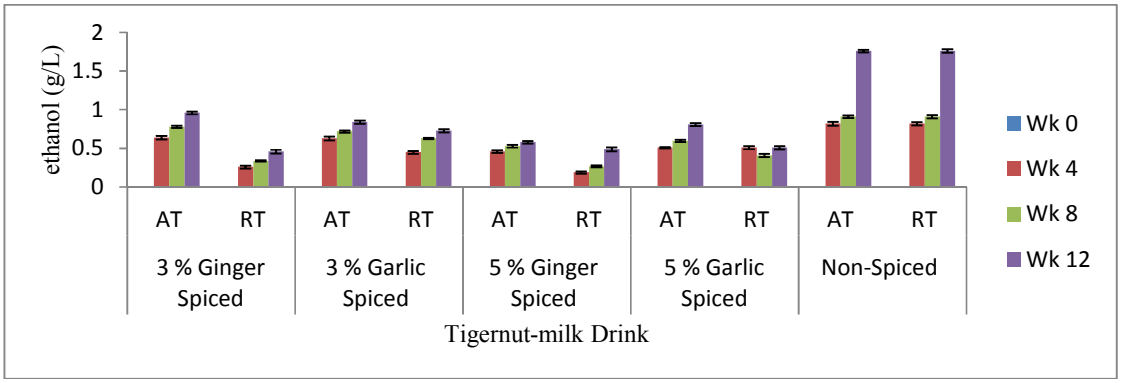

Fig. 1. Ethanol content of spicy and non-spicy lactic fermented tigernut-milk drink during storage at ambient and refrigeration temperature. 'AT' depict ambient temperature (28±2°C); 'RT' depict refrigeration temperature (4±2°C)

100 ml lactic fermented tigernut milk drinks spiced with ginger and garlic stored at ambient temperature and a similar drink stored at refrigeration temperature except 3% garlic spiced lactic fermented tigernut milk drink at Week 0. This result trend indicates that both storage conditions influenced the calorie content of ginger and garlic spiced lactic fermented tigernut-milk drinks.

Despite the reduction in calorie content of spicy lactic fermented tigernut-milk drinks during storage at both ambient (28±2°C) and refrigeration temperature (4±2°C), the remaining calorie content in garlic and ginger spiced lactic fermented tigernut-milk drinks was still adequate to meet the energy need of consumers based on the official conversion factor adopted by Council Directive on Nutrition Labeling of Foodstuffs possibly as a result of high carbohydrate and fat content of spicy lactic fermented tigernut-milk drink. It could also be that addition of ginger and garlic to the lactic fermented tigernut-milk drink provided additional calorie to balance the loss in calorie content of the drink as a result of breakdown of protein, fat and sugar. The range of energy value of ten varieties of ginger reported by [30] is between 374.96-396.17 Kcal/100 g. Eleazu [31] reported that the energy value of freshly peeled garlic cloves was 145.00 Kcal. Papu [32] reported a lower energy value (119 Kcal/100 g) in garlic compared with higher energy value in ginger (374.96-396.17 Kcal/100 g). According to United States Department of Agriculture (USDA), the food energy in beverages or soft drinks in terms of allowed Recommended Daily Allotments (RDA) should not be more than ten spoons for 2000 calories diet [5]. The energy value of tigernut-milk is approximately 100 cal/100 g. It is interesting to report that the energy value of spicy lactic fermented tigernut-milk drinks is higher than tigernut-milk drink. Therefore, spicy lactic fermented tigernut-milk drink can be considered as a natural energetic drink recommended to everyone [10].

Table 2 depicts the total soluble solids in ginger and garlic spiced lactic fermented tigernut-milk drinks stored at ambient (28±2°C) and refrigeration (4±2°C) temperature for 12 Weeks. This study revealed that total soluble solids in spicy lactic fermented tigernut-milk drinks stored at refrigeration temperature was higher than spicy lactic fermented tigernut drinks stored at ambient temperature with few exceptions during storage of 5% ginger spiced lactic fermented tigernut-milk drink. This observation could be as a result of higher bacterial population in lactic fermented tigernut-milk drinks spiced with ginger and garlic which were stored at ambient temperature compared with similar drinks stored at refrigeration temperature. In a related study carried out by [23], they reported that lactic acid bacterial population in spiced and non-spiced lactic fermented tigernut-milk drinks stored at ambient temperature (28±2°C) was higher than lactic acid bacterial population in similar drinks stored at refrigeration temperature (4±2°C) with very few exceptions. The involvement of high bacterial population in a fermentation process results in fast utilization of available sugar for metabolic activities thereby reducing the total soluble solids in the fermented product [29]. Falade [33] in a related study reported that total soluble solids in yoghurt decreased with increased temperature of storage and was attributed to higher microbial activities in the product because of higher storage temperature.

Table 1. Calorific value per 100 ml spicy lactic fermented tigernut-milk drink during storage at ambient and refrigeration temperature

Wk	3% Ginger Spiced Tigernut-milk Drink		3% Garlic Spiced Tigernut-milk Drink		5% Ginger Spiced Tigernut-milk Drink		5% Garlic Spiced Tigernut-milk Drink	
	AT	RT	AT	RT	AT	RT	AT	RT
				Kcal				
0	200.48±0.594^{d}	191.84±0.431^{d}	177.35±0.473^{d}	177.58±0.438^{d}	245.08±0.976^{d}	230.21±0.948^{d}	179.93±0.198^{d}	180.17±0.318^{d}
4	183.50±0.608^{c}	190.39±0.636^{c}	149.22±0.382^{c}	146.28±0.516^{b}	216.79±0.438^{c}	207.91±0.587^{c}	170.85±0.735^{c}	157.69±0.460^{c}
8	162.22±0.537^{b}	173.21±0.325^{b}	135.68±0.764^{a}	155.47±0.757^{c}	202.49±0.566^{b}	200.46±0.290^{b}	134.41±0.785^{a}	141.71±0.460^{a}
12	148.72±0.417^{a}	159.39±0.544^{a}	144.73±0.530^{b}	140.58±0.488^{a}	185.86±0.266^{a}	187.58±0.792^{a}	137.88±0.262^{b}	155.07±0.962^{b}

Values show means of duplicate analysis ±SD. Figures with different superscript down the colunm, are significantly different (P<0.05).
'AT' depict ambient temperature (28±2°C); 'RT' depict refrigeration temperature (4±2°C)

Table 2. Total soluble solids in spicy lactic fermented tigernut-milk drink during storage at ambient and refrigeration temperature

Wk	3% Ginger Spiced Tigernut-milk Drink		3% Garlic Spiced Tigernut-milk Drink		5% Ginger Spiced Tigernut-milk Drink		5% Garlic Spiced Tigernut-milk Drink	
	AT	RT	AT	RT	AT	RT	AT	RT
				°B				
0	11.39±0.750^{c}	12.95±0.247^{c}	13.18±0.544^{c}	13.24±0.403^{d}	14.31±0.481^{c}	11.76±0.255^{c}	13.30±0.933^{b}	13.40±0.530^{b}
4	8.72±0.693^{b}	9.16±0.559^{b}	7.57±0.396^{b}	11.86±0.262^{c}	11.71±0.955^{b}	10.21±0.629^{b}	10.88±0.841^{ab}	11.78±0.438^{ab}
8	8.11±0.488^{b}	8.81±0.516^{ab}	7.27±0.870^{b}	9.43±0.403^{b}	10.55±0.573^{ab}	9.92±0.792^{ab}	9.41±0.502^{a}	10.77±0.877^{a}
12	5.54±0.658^{a}	7.42±0.658^{a}	5.29±0.233^{a}	8.35±0.368^{a}	8.64±0.820^{a}	8.65±0.573^{a}	8.30±0.651^{a}	10.50±0.643^{a}

Values show means of duplicate analysis ±SD. Figures with different superscript down the colunm, are significantly different (P<0.05).
'AT' depict ambient temperature (28±2°C); 'RT' depict refrigeration temperature (4±2°C)

According to [34], addition of spices such as ginger to fermented drinks could reduce the amount of sugar in the spiced drinks. In their study, [34] reported that total soluble solids in spiced fruit drink reduced with increasing amount of spices added to the drink. However, this study revealed that total soluble solids in ginger and garlic spiced lactic fermented tigernut-milk drinks increased with increased concentration of each spice added to the drinks. This could be as a result of the variety of ginger and garlic used to spice lactic fermented tigernut-milk drink [31,35]. According to [36], large quantity of total soluble solids in a fermented beverage is an indication that the product is safe for human consumption pending other confirmatory tests.

The reduction in total soluble solids with increase in fermentation time reported in this study is in agreement with a similar study carried out by [37] which involved fermentation and storage of a probiotic beverage. A similar result trend was also reported by [38] during fermentation of an alcoholic beverage. According to the research findings of [39], soluble solids in soy-yoghurt decreased during the period the product was stored at 6°C. They attributed their research findings to be as a result of metabolism of sugars by lactic culture present in the product during the period of storage. Singh [40] reported that the total soluble solids in tigernut beverages ranged between 11.23-14.27 °B. Their study revealed that addition of *Hibscus sabdariffa* extract (HSE) and *Moringa oleifera* to tigernut-milk drink spiced with ginger (*Zingiber officinale*) did not cause significant changes in the total soluble solids in tigernut-milk drinks. In a related study, [29] reported that percentage total solids in fermented tigernut-milk drink reduced during the period of storage which they attributed to microorganisms that gradually utilized milk solids and converted the substrate to non-solid products. However, [41] reported that total soluble solids in tigernut-milk drink spiced with some flavouring agents increased during the period of storage of the product with few exceptions.

4. CONCLUSION

This study investigated the effects of adding ginger and garlic to lactic fermented tigernut-milk on the loss of its calorie content and overall quality during storage at ambient (28±2°C) and refrigeration (4±2°C) temperature. In the course of storing, there was a significant reduction in total soluble solids, but a less significant reduction in calorie content of the ginger and garlic spiced lactic fermented tigernut-milk drinks, except the calorie content of 3% garlic spiced drink stored at refrigeration temperature from four to eight weeks. In addition, fermentation gradually increased ethanol content of the spiced fermented drinks, which was however, evidently lower than that in non-spiced drink throughout the period of storage.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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